ORIGINAL ARTICLE

Testing for reproductive interference in the population dynamics of two congeneric species of herbivorous mites

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When phylogenetically close, two competing species may reproductively interfere, and thereby affect their population dynamics. We tested for reproductive interference (RI) between two congeneric haplo-diploid spider mites, *Tetranychus evansi* and *Tetranychus urticae*, by investigating their interspecific mating and their population dynamics when they competed on the same plants. They are both pests of tomato, but differ in the host plant defences that they suppress or induce. To reduce the effect of plant-mediated interaction, we used a mutant tomato plant lacking jasmonate-mediated anti-herbivore defences in the competition experiment. In addition, to manipulate the effect of RI, we introduced founder females already mated with conspecific males in mild RI treatments or founder, virgin females in strong RI treatments (in either case together with heterospecific and conspecific males). As females show first-male sperm precedence, RI should occur especially in the founder generation under strong RI treatments. We found that *T. urticae* outcompeted *T. evansi* in mild, but not in strong RI treatments. Thus, *T. evansi* interfered reproductively with *T. urticae*. This result was supported by crossing experiments showing frequent interspecific copulations, strong postmating reproductive isolation and a preference of *T. evansi* males to mate with *T. urticae* (instead of conspecific) females, whereas *T. urticae* males preferred conspecific females. We conclude that interspecific mating comes at a cost due to asymmetric mate preferences of males. Because RI by *T. evansi* can improve its competitiveness to *T. urticae*, we propose that RI partly explains why *T. evansi* became invasive in Europe where *T. urticae* is endemic. *Heredity* (2014) **113**, 495–502; doi:10.1038/hdy.2014.53; published online 28 May 2014

INTRODUCTION

In some animal species, males readily mate with heterospecific females, supposedly because they are unable to discriminate between the species (Gröning and Hochkirch, 2008), and they may even prefer to mate with heterospecific females instead of conspecific females (for example, Ludden *et al.*, 2004; Hochkirch *et al.*, 2008). This may come at a cost to the females because it can hamper or even block their reproduction and it may even cause populations of their species to be outcompeted. Thus, reproductive interference may alter the species composition in a community (Kuno, 1992), but such an effect can be brought about by other competitive processes. For example, communities of plant-inhabiting arthropods are not only determined by direct competition, but also by indirect modes of competition, such as plant-mediated competition (Denno *et al.*, 1995; Ohgushi, 2005; Denno and Kaplan, 2007) and predator-mediated competition (Holt, 1977; Holt and Lawton, 1994; van Veen *et al.*, 2006). Hence, to test for the occurrence of reproductive interference (RI) between species and its effect on the outcome of the interspecific competition, it is necessary not only to perform crossing experiments between (closely related) species and to compare the population dynamics in presence and absence of other species in the field (Gröning and Hochkirch, 2008), but also to carefully design population experiments in which other competitive processes, such as plant-mediated and predator-mediated competition, are excluded and in which the effect of RI is manipulated (for example, Kishi *et al.*, 2009).

To assess the role of RI, we studied two congeneric haplo-diploid species of spider mites, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) and *Tetranychus urticae* (Koch) (Acari: Tetranychidae) (Figure 1). Both species are important pests of solanaceous crops, especially of tomato plants (*Solanum lycopersicum* L.), the first species being a host plant specialist and the second being a generalist (Helle and Sabelis, 1985; Navajas *et al.*, 2012). Each of the two species spins a protective web on the leaves of their host plant, and under such webs groups of spider mites feed, mate, reproduce and develop. Before mating males guard the last moulting stage of the females and mate directly upon emergence of the female from the exuvium. Proximity to the emerging female is an important determinant of male mating success (for example, Potter *et al.* 1976). *Tetranychus evansi* from South America has become an invasive species first in Africa and then in Europe (Boubou *et al.*, 2012; Navajas *et al.*, 2012). Nowadays, its distribution overlaps with that of the closely related species *T. urticae* in these regions (Helle and Sabelis, 1985; Navajas *et al.*, 2012). Hence, these two species are expected to interact with each other when sharing host plants in the field. Both species belong to the same genus *Tetranychus*, and in spider mites, RI has been observed between species from the same or even of different genera (Fujimoto *et al.*, 1996; Takafuji *et al.*, 1997; Navajas *et al.*, 2000; Ben-David *et al.*, 2009). RI is, therefore, likely to affect the outcome of competition between *T. evansi* and *T. urticae*.

Using these two species of spider mites provides us with advantages in designing a competition experiment that allows testing for the

Figure 1 Photos of two species of spider mites, (a) *Tetranychus urticae* and (b) *Tetranychus evansi*, that we used in the experiments. Both species are pest of tomato plants but there is a difference in the colour of the soma (thorax, plus abdomen): green or whitish for *T. urticae* and red for *T. evansi*. These colours stem from pigment in the haemolymph and internal tissues (not the cuticle or the internal gut lumen).

effect of RI on spider mite dynamics. First, spider mites show strong first-male sperm precedence (Boudreaux, 1963; Helle, 1967), indicating that secondary matings are less effective and that females do not exhibit cryptic male choice by selecting among sperm of various males for fertilisation of their eggs. This allowed us to manipulate the founder females that were introduced to the competition experiment as follows. In one experiment, we created a mild RI treatment in the founder generation by releasing heterospecific and conspecific males with females that had previously mated with conspecific males. These females can, therefore, reproduce irrespective of secondary matings with heterospecific males. In a parallel experiment, we created a strong RI treatment in the founder generation by releasing heterospecific and conspecific males with virgin females, which can then mate with conspecific and heterospecific males, the latter of which can potentially result in decreased reproduction. A second advantage of using the two species of spider mites is that there is much knowledge of the impact they have on host plant defences: within leaves of tomato plants many strains of *T. urticae* induce the jasmonate(JA)-mediated plant defences (Li *et al.*, 2002; Ament *et al.*, 2004; Kant *et al.*, 2004, 2008), whereas *T. evansi* downregulates these defences, even below household levels (Sarmiento *et al.*, 2011a), thereby making the host plant more profitable for both *T. evansi* and *T. urticae* (Sarmiento *et al.*, 2011b). By using mutant *def-1* tomato plants that are deficient in mounting JA-mediated plant defences (Howe *et al.*, 1996; Li *et al.*, 2002; Ament *et al.*, 2004; Kant *et al.*, 2008), we were able to reduce the effect of JA-mediated plant defences in our competition experiments, thereby gaining more analytic power to detect RI. Also, we were able to exclude predator-mediated competition by introducing only the two spider mite species on the plants in an isolated environment (climate room).

Hence, by taking advantage of these features from our experimental system, we studied the effect of RI on the competition between *T. evansi* and *T. urticae*. This was done by comparing the population growth of the two species between experiments initiated by founder females differing in reproductive status (virgin or mated with conspecific males) on mutant *def-1* tomato plants and in absence of natural enemies of the spider mites. We also investigated their mating behaviour, their reproductive compatibility, and male mate choice when males were offered females of these two species simultaneously, in order to analyse the results from our competition experiments.

MATERIALS AND METHODS

Plants

Tomato seeds (*S. lycopersicum* cv. Castlemart) were sown in 12-cm pots and watered two times per week. After maintaining the plants in a greenhouse for 3 weeks, they were transferred to a climate chamber (25 °C; 60% relative humidity (RH); 16:8 h light:dark photoperiod). When the tomato plants were 4–5-week-old, leaves were detached and then used to maintain the colonies. The interspecific competition experiments were carried on *def-1* mutant tomato plants that have a Castlemart genetic background (Howe *et al.*, 1996). These *def-1* plants were also grown from seeds and maintained in the same way as the Castlemart plants. We used this plant genotype because the *def-1* mutation impairs the accumulation of JA and thereby also the JA-mediated plant defence mechanisms induced after wounding by herbivorous arthropods (Howe *et al.*, 1996). In this way, we reduced the effect of plant-mediated competition between the two phytophagous species.

Mites

Tetranychus urticae and *T. evansi* were collected from *S. nigrum* L. in Málaga (N36°34'29", W5°57'35"), Spain, August 2010. Harvested plant material was placed in separate zip-bags and transported to Amsterdam. All adult females died during transport, however, there were still mites in different developmental stages on the sampled leaves. Subsequently the plant material was transferred to separate trays with tomato leaves and incubated in a quarantine climate box to allow mites to complete the development (25 °C; 60% RH; 16:8 h light:dark photoperiod). These quarantine cultures were checked every 2 days and adult females were transferred to clean tomato leaves and moved to a climate room providing the same conditions. When the colonies were established, 10 males per colony were sampled and placed in 70% ethanol. The species identity of both strains was confirmed on the basis of the aedeagus (the male reproductive organ) morphology (Gutierrez and Etienne, 1986). Whereas *T. evansi* was reared on detached tomato leaves (cv. Castlemart), *T. urticae* was reared on detached common bean leaves (*Phaseolus vulgaris* L.) on wet cotton wool in a plastic box under constant climatic conditions (25 °C; 60% RH; 16:8 h light:dark photoperiod). At least 1 month before use in the experiments, *T. urticae* was moved to detached tomato leaves under the same climatic conditions.

Females of the two species to be used for the experiments were collected in the teleiochrysalis stage (i.e. moulting stage preceding adult phase; thus definitely before mating occurs immediately after adult emergence) so that they were virgins after their last moult. Hence, we could always exactly infer their mating status from subsequent exposure to males. Males of the two species were obtained from eggs laid by virgin females. Because spider mites reproduce by arrhenotokous parthenogenesis, virgin females produce haploid eggs that develop into males (Helle and Sabelis, 1985).

Crossing experiments

Leaf discs (1.5 cm in diameter), punched from tomato plants (4–5 week-old), were placed on wet cotton wool in a Petri dish, and used as an arena for either

mating or rearing of offspring. To observe premating behaviour of spider mites, a single teliochrysalis female was placed onto a leaf disc where it moulted into the adult phase within 24 h. Subsequently, three virgin males, 1–7-day-old since their last moult, were introduced for mating. The occurrence and duration of copulations was recorded for 0.5 h using a camera (Leica IC80 HD, Leica Microsystems GmbH, Germany) connected to a binocular microscope and a computer with adequate software (LAS EZ software version 2.0.0, Leica Microsystems GmbH). In an independent experiment, we assessed reproductive compatibility between females and males in exactly the same set-up (one female, three males, leaf disc). They were allowed to copulate and oviposit for 5 days. Egg and juvenile survival was checked every 48 h, and gender was checked when they developed into adults. The leaf discs were replaced with fresh ones every 4 days. Using this set-up, we carried out mating and crossing experiments between *T. urticae* and *T. evansi*, and also within each of the two species as a control. In addition, we assessed reproduction by virgin females of each of the two species. We evaluated the compatibility of *T. urticae* and *T. evansi* by comparing the number of eggs deposited, the offspring survival and the sex ratio (% females) between intraspecific and interspecific crosses. Prezygotic reproductive barriers were inferred from differences in sex ratios, whereas postzygotic barriers were inferred from juvenile survival. The crossing experiments were all performed under constant climatic conditions (25 °C; 60% RH; 16:8 h light:dark photoperiod).

Male mate choice

In spider mites, females have a preference when given a choice between males differing in relatedness and bacterial infection status (Vala *et al.*, 2004; Tien *et al.*, 2011). However, females usually cannot express this preference because males guarding females in the teliochrysalis stage immediately mate upon female emergence (Potter *et al.*, 1976). We, therefore, assumed that male mate choice prevails. A teliochrysalis female of *T. evansi* and one of *T. urticae* were introduced on a leaf disc (1.5 cm). After the two females moulted to the adult phase, one *T. evansi* male or one *T. urticae* male, moulted 2 days earlier, were released on the leaf arena. Male behaviour was observed until copulation occurred. The pairs in which copulation did not occur within 0.5 h after male introduction were excluded from further analysis. This experiment was carried out under constant climatic conditions (25 °C; 60% RH; 16: 8 h light:dark photoperiod).

Interspecific competition on plants

To investigate the competitive relation between the two species, we observed population growth of the mites over a period of 4 weeks (2–3 generations) for the case where two species were introduced together on a tomato plant and for the case where each of the two species mites were introduced separately on a tomato plant. To detect the effect of RI, we modified the reproductive status of founder females in two treatments: they were either virgin or mated with conspecific males. The experimental design is summarized in Table 1. As the spider mites increase exponentially and as they, by doing so, cause death of the tomato plant within a few weeks, we introduced a small number of mites (four

females and four males) on a tomato plant as founders. To reduce the effect of JA-mediated plant defence, we used an intact *def-1* tomato plant as the host plant. Details of the process of the experiment were as follows.

Four females and four males were released together on a leaflet of the third or fourth leaf from an intact *def-1* tomato plant (3-week-old). Their establishment on the leaflet was checked for 3 days after mite introduction. When mites were missing during these first 3 days (for example, by accidental death or by falling down), they were replaced to ensure a fixed number of founder mites (thereby reducing the probability that small initial differences in numbers were magnified through exponential growth).

We carried out six treatments, differing in species composition (*T. urticae*, *T. evansi* or 'mix') and the mating status of founder females ('virgin' or 'mated') (Table 1). In the first treatment (mix, virgin), two females and two males of each species were released, of which the females were virgin. This served to assess competition under conditions where RI could operate strongly from the start of the experiment (strong RI treatment). To obtain weak RI, we released two mated females and two males of each species (mix, mated; mild RI treatment). Here, RI could mainly arise among the offspring of the mated females. A further four treatments served as controls and consisted of either four mated or four virgin females and four males of each species separately (*T. urticae* virgin, *T. urticae* mated, *T. evansi* virgin and *T. evansi* mated). In all treatments, females and males were 2-day-old since they reached adulthood. To obtain mated females, 1-day-old virgin females were introduced on leaf arenas with males of the same species, and they were kept together for 1 day to give them sufficient opportunity to copulate. The ratio of males and females on each mating arena was 1:2.

After releasing the mites on the plant, the adult females of each species were counted once per week over a period of four weeks. Females of the two species were differentiated based on their body colour (resulting from pigments in the haemolymph and internal tissue, not in the cuticle or the gut) (Figure 1). The experiments were carried out under constant climatic conditions (25 °C; 60% RH; 16:8 h light:dark photoperiod).

Statistical analysis

The number of pairs where copulation occurred was compared between intra- and interspecific crossings for each of the two species of males, using Fisher's exact test for count data. To test for effects of the species of the male (*T. urticae* or *T. evansi*) and crossing type (intra- or interspecific) on the duration of copulation (s), we used a two-way analysis of variance (two-way ANOVA). To test for effects of female species (*T. urticae* or *T. evansi*) and crossing type (intra- or interspecific) on the number of eggs, we also used a two-way ANOVA. Juvenile survival rate and offspring sex ratio were analysed using generalised linear models with a binomial error distribution using the species of the female (*T. urticae* or *T. evansi*) and mating type (intra- or interspecific) as factors. The effect of each factor was assessed with likelihood ratio tests, comparing the saturated model with the model without the factor.

Male mate choice was analysed using exact binomial tests for each species, taking the probability to choose a conspecific female equal to 0.5 under the null hypothesis.

Table 1 Design of the interspecific competition experiment between *T. urticae* and *T. evansi* on tomato plants

Treatment	Strong RI	Mild RI	Control1	Control 2	Control 3	Control 4
Founders	Mix, virgin	Mix, mated	<i>T. urticae</i> virgin	<i>T. evansi</i> virgin	<i>T. urticae</i> mated	<i>T. evansi</i> mated
Female						
<i>T. urticae</i>	2 virgin	2 mated	4 virgin	—	4 mated	—
<i>T. evansi</i>	2 virgin	2 mated	—	4 virgin	—	4 mated
Male						
<i>T. urticae</i>	2 virgin	2 virgin	4 virgin	—	4 virgin	—
<i>T. evansi</i>	2 virgin	2 virgin	—	4 virgin	—	4 virgin

Abbreviation: RI, reproductive interference.

Six treatments, each replicated six times, were done with various combinations of virgin or mated females and virgin males with the two different species (*T. urticae* or *T. evansi*) at the start of the experiments. Numbers in the body of the table refer to the number of individuals released.

For the analysis of interspecific competition on plants, generalised linear mixed-effects models (with a Poisson error distribution, 'glmer' in package 'lme4' from the statistical package R) were used because repeated measurements were taken over time. To compare the growth curve (change of the number of females over time on a plant) among the control treatments (i.e. in the absence of interspecific competition), a model was constructed with species ('*T. urticae*' or '*T. evansi*'), the reproductive status ('virgin' or 'mated'), time (weeks after mite introduction) and their interactions as fixed factors and with replicate as random factor. To compare the growth curve between strong RI treatment (mix, virgin) and mild RI treatment (mix, mated), a model was constructed with species ('*T. urticae*' or '*T. evansi*'), the reproductive status ('virgin' or 'mated'), time (weeks after mite introduction) and their interactions as fixed factors and with replicate as random factor. As we want to know if RI affects the competition (the relation of growth curves) between *T. urticae* and *T. evansi*, we tested the effect of higher-order interaction (species $\times$ time $\times$ reproductive status) on the number of female mites in the latter model. If the competition between *T. urticae* and *T. evansi* changes depending on the reproductive status (virgin or mated), the higher-order interaction (species $\times$ time $\times$ reproductive status) should be significant. To confirm if simply the reproductive status of the founder females affects the relation of growth curve between these two species, we tested the effect of the higher-order interaction in the former model. In the tests, we compared the saturated model with the model without higher-order interaction by a likelihood ratio test. We followed the procedure described by Faraway (2006) in constructing these models. The analyses were performed with the statistical package R ver. 2.14.2 (R Development Core Team (2011)).

RESULTS

Crossing experiment

Interspecific copulation occurred between females of *T. urticae* and males of *T. evansi* as well as between males and females of the respective species, although the frequency of copulation of *T. urticae* males with *T. evansi* females was significantly lower than that of intraspecific copulations in *T. urticae* (Fisher's exact test, *T. urticae* ($\varnothing$) $\times$ *T. urticae* (σ) vs *T. evansi* ($\varnothing$) $\times$ *T. urticae* (σ): $P=0.024$, *T. evansi* ($\varnothing$) $\times$ *T. evansi* (σ) vs *T. urticae* ($\varnothing$) $\times$ *T. evansi* (σ): $P=1.000$; Figure 2). The duration of the copulation in interspecific combinations was 2.5 times shorter than that in intraspecific pairs and this difference was significant (two-way ANOVA, crossing type: $F_{1,122}=340.44$, $P<0.001$, male species: $F_{1,122}=3.65$, $P=0.06$; Figure 3). The number of eggs produced after interspecific crossings did not differ significantly from that after intraspecific crossings, although the number of eggs laid by *T. urticae* females was significantly larger than that by *T. evansi* (two-way ANOVA, crossing type: $F_{1,142}=0.83$, $P=0.36$, female species: $F_{1,142}=4.33$, $P=0.039$; Figure 4a). The juvenile survival rate of offspring from interspecific crossings was lower than that from intraspecific crosses, but this effect was bordering significance (likelihood ratio test, $\chi^2=3.68$, $P=0.06$; Figure 4b). The effect of female species on the juvenile survival rate of offspring was not significant (likelihood ratio test, $\chi^2=0.003$, $P=0.96$; Figure 3b). Unmated females and females of interspecific crossings produced predominantly male offspring, but females from intraspecific crosses produced female-biased offspring (likelihood ratio test, cross type: $\chi^2=983.19$, $P<0.001$, female species: $\chi^2=0.16$, $P=0.69$; Figure 4c). Only a single replicate of the crossing between *T. evansi* females and *T. urticae* males yielded three females, which represented genuine hybrids (Figure 4c).

Male mate choice

Most *T. urticae* males first copulated with conspecific females (exact binomial test, $P<0.001$; Figure 5). However, most *T. evansi* males first copulated with *T. urticae* females rather than with conspecific females (exact binomial test, $P<0.001$; Figure 5).

Figure 2 The fraction of replicates in which intra- or interspecific copulations occurred within 0.5 h after introduction of a virgin female and three virgin males onto a tomato leaf arena. Asterisks indicate statistically significant differences (Fisher's exact test, $*P<0.05$).

Figure 3 Bar plots of the duration of intra- or interspecific copulations. Number of replicates are given above the column bars expressing the means, whereas the s.e.m are given as vertical line bars. The effect of crossing type on the duration of copulations was significant, and the effect of species of male was bordering significance (two-way ANOVA, crossing type: $F_{1,122}=340.44$, $P<0.001$, male species: $F_{1,122}=3.65$, $P=0.06$).

Competition on plants

In treatments where each of the two species was introduced separately on a *def-1* tomato plant (controls; Table 1), the number of females

Figure 4 Bar plots of (a) the number of eggs, (b) the juvenile survival rate of offspring and (c) the female sex ratio among offspring produced by females mated with conspecific or heterospecific males or by virgin females. Column bars express means and vertical line bars represent standard errors. The effect of crossing type on the number of eggs was not significant (two-way ANOVA, crossing type: $F_{1,142}=0.83$, $P=0.36$, female species: $F_{1,142}=4.33$, $P=0.039$). However, the effect of crossing type on the juvenile survival rate of offspring was bordering significance (likelihood ratio test, cross type: $\chi^2=3.68$, $P=0.06$, female species: $\chi^2=0.003$, $P=0.96$), and the effect of crossing type on the sex ratio of offspring was significant (likelihood ratio test, cross type: $\chi^2=983.19$, $P<0.001$, female species: $\chi^2=0.16$, $P=0.69$).

Figure 5 The fraction of *T. urticae* (gray bar) or *T. evansi* (white bar) females that *T. urticae* (upper bar) and *T. evansi* (lower bar) males selected for the first mating after being introduced onto a leaf arena with one *T. urticae* and one *T. evansi* female together (exact binomial test, *** $P<0.001$).

increased with time and the growth rate of *T. evansi* was higher than that of *T. urticae*. The difference between growth curves of *T. urticae* and *T. evansi* did not depend on the reproductive status, as the

interaction between species, time and reproductive status did not have a significant effect (likelihood ratio test, species $\times$ reproductive status $\times$ time: $\chi^2=0.645$, $df=1$, $P=0.422$; Figures 6c and d).

When the two species were introduced together on the same plants (treatments of strong RI and mild RI; Table 1), there was a significant effect in the interaction between species, reproductive status and time (likelihood ratio test, species $\times$ reproductive status $\times$ time: $\chi^2=19.261$, $df=1$, $P<0.001$; Figures 6a and b). This means that the competitive relation between *T. urticae* and *T. evansi* (the difference between the growth curves of *T. urticae* and *T. evansi*) significantly differs between strong and mild RI treatments (Figures 6a and b).

DISCUSSION

We found that under strong RI treatment, the colony growth of *T. evansi* was similar to that of *T. urticae* (Figure 6a), whereas under mild RI treatment, *T. evansi* was inferior to *T. urticae* (Figure 6b). These results suggest that *T. evansi* counteracted *T. urticae* by interfering with the reproduction of this competitor. This inference is supported by the results of crossing experiments and male mate choice experiments: interspecific copulation readily occurred between these two species (Figures 2 and 3), they are reproductively

Figure 6 Population dynamics of *T. urticae* and *T. evansi* on tomato plants (*def-1*) over a period of 4 weeks after mite introduction in strong RI treatment (a), mild RI treatment (b), controls of strong RI treatment (controls 1 and 2) (c), and controls of mild RI treatment (controls 3 and 4) (d). For the mite release design, see Table 1. Filled circles indicate the mean number of *T. evansi* adult females per founder female, and open circles indicate the mean number of *T. urticae* adult females per founder female. Vertical bars indicate s.e.m. The number of replicates per treatment is six. When both species were released on a tomato plant together (panels a and b), the interaction between species, reproductive status and time had a significant effect on the number of female mites (likelihood ratio test, species $\times$ reproductive status $\times$ time: $\chi^2 = 19.261$, $df = 1$, $P < 0.001$). However, when these species were released on a tomato plant separately (panels c and d), the effect of the interaction was not significant (likelihood ratio test, species $\times$ reproductive status $\times$ time: $\chi^2 = 0.645$, $df = 1$, $P = 0.422$).

incompatible (postmating, prezygotic reproductive barriers; Figure 4) and *T. evansi* males preferred *T. urticae* females to conspecific females, whereas *T. urticae* males preferred conspecific females to *T. evansi* females (Figure 5).

Given that *T. evansi* suppresses and *T. urticae* induces host plant defences, the infestation of the plants by *T. evansi* makes the host plant more profitable for *T. urticae* (Sarmiento *et al.*, 2011b). Indeed, earlier experiments showed that the oviposition rate of *T. urticae* increased on tomato leaves previously infested by *T. evansi*, whereas *T. evansi* showed a decrease in oviposition on leaves previously infested by *T. urticae* (Sarmiento *et al.*, 2011a). Thus, their co-occurrence on tomato plants may benefit *T. urticae* but comes at a cost to *T. evansi*. Nevertheless, *T. evansi* was found to outcompete *T. urticae* (Sarmiento *et al.*, 2011b). Moreover, field observations in Spain showed that *T. evansi* invasion replaced native *Tetranychus* species including *T. urticae* (Ferragut *et al.*, 2013). Hence, given that *T. evansi* suppresses tomato plant defence, the question arises how *T. evansi* prevents *T. urticae* to profit from this. One explanation for the successful invasion of *T. evansi* into Europe is that it can prevent *T. urticae* from taking advantage of the suppressed plant defences by covering the exploited leaf area with a more dense web than that of *T. urticae* and by producing even more web in response to *T. urticae*, thereby reducing access of this competitor to the leaf covered by

T. evansi web (Sarmiento *et al.*, 2011b). However, the web produced by *T. evansi* may be more effective when populations are large (note that both species produce web and live under the web) and it may still be questioned to what extent it has a role at low mite densities. We suggest that other processes, such as RI, shown in this article, may explain why *T. evansi* prevents *T. urticae* from profiting from the *T. evansi*-induced suppression of plant defences and also why *T. evansi* succeeded in invading and displacing other *Tetranychus* species already established. RI likely works against invasive species, because colonisation of invasive species typically starts from small number of founders (Sakai *et al.*, 2001), especially when the introduction occurs accidentally by human activity as in the case of *T. evansi* (Boubou *et al.*, 2012). RI is frequency dependent and the majority has an advantage when inter-sexual interactions between species are reciprocal (Kuno, 1992). However, most of the inter-sexual interactions between species are asymmetric (Gröning and Hochkirch, 2008), and this can allow invasive species to overcome the disadvantage of small colony size. Recently, biotype B of the whitefly, *Bemisia tabaci*, invaded and displaced indigenous biotypes of the species in Zhejiang (China; indigenous biotype is ZHJ1) and Queensland (Australia; indigenous biotype is AN; Liu *et al.*, 2007). Behavioural observations revealed asymmetric mating interactions between invasive and indigenous biotypes: when the proportion of males in the offspring

increased by adding males of the same or different biotype to a pair, the pair of biotype B increased the number of copulations and consequently increased the proportion of female progeny regardless of the biotype of additional males, however, the pair of indigenous biotypes (ZHJ1 and AN) decreased the number of copulations and the proportion of female progeny when additional males were biotype B (Liu *et al.*, 2007). This asymmetric mating interaction and the capacity to adjust the sex ratio in favour of the population increase in biotype B are expected to be key features of biotype B driving its widespread invasions and displacement, and this expectation is supported by long-term field surveys and population experiments in cages (Liu *et al.*, 2007). In our study, *T. evansi* males showed mating preference to *T. urticae* females, whereas *T. urticae* males prefer conspecific females. It is unknown why *T. evansi* males prefer females of *T. urticae*. We hypothesize that *T. urticae* females produce more of a similar sex pheromone to attract males near emergence from the last moulting stage. Whatever the explanation may be, the preference of *T. evansi* males to copulate with *T. urticae* females is possibly one of the key features of *T. evansi* driving its invasion and displacement in areas endemic to *T. urticae*.

To exclude or reduce indirect plant-mediated interactions between the two mite species via mite-induced plant defences, we used a *def-1* tomato mutant that is deficient in JA accumulation after wounding and hence lacks the expression of genes in response to increased JA. In doing so, we expected that *T. urticae* could not benefit from the suppressed plant defences induced by *T. evansi* on the same leaves and that *T. evansi* would not incur a cost by the plant defences induced by *T. urticae*. However, the results did not completely meet our expectations. Comparing the population dynamics of *T. evansi* under mild RI treatment (Figure 6b) and the control (*T. evansi*, mated; Figure 6d), it can be seen that the population growth of *T. evansi* was hampered by the presence of *T. urticae*. In contrast, the same comparison for *T. urticae* shows that colony development of *T. urticae* was promoted by the presence of *T. evansi* (mild RI: Figure 6b; *T. urticae*, mated: Figure 6d). This suggests that *T. urticae* induces plant defences that hamper the reproductive performance of other spider mites, not only through JA signalling. Previous studies showed higher reproductive performance of spider mites on a *def-1* tomato mutant compared with a wild tomato (Li *et al.*, 2002; Ament *et al.*, 2004), but recent studies show circumstantial evidence for anti-herbivore defences mediated by salicylic acid, another signal transducer mediating the expression of plant defence genes (Farouk and Osman, 2011, but see Kawazu *et al.*, 2011). Although we recognize the possibility of a role for plant defences that are not mediated by JA, we emphasize that this does not change our conclusion regarding the impact of RI. We expect RI to be much more common than shown so far, if future experiments are based on experimental designs that exclude other factors (such as plant defence and shared natural enemies).

DATA ARCHIVING

Data available from the Dryad Digital Repository: doi:10.5061/dryad.2n85d.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank Dr Merijn R Kant, Dr Livia Silva Ataíde and Mrs Dan Li from the University of Amsterdam for their useful suggestions on the study of interspecific competition on plants and Dr Michiel van Wijk for his help in

setting up the video recording equipment. Dr Arne Janssen and Dr Sara Magalhães provided many useful comments on a first draft of the manuscript. Jan van Arkel made the excellent photos of the two mite species shown in Figure 1. JMA was funded as a postdoc via NWO Earth and Life Sciences (ALW) TOP (854.11.005) and YS was funded as a postdoc via the budget of MWS for his Royal Academy of Sciences (KNAW) professorship (selected in 2006 for 5 years and prolonged from 2012 to 2015).

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